STANFORD UNIVERSITY LIBRARY
DEC 10 1928

A STUDY OF THERMOPHILIC BACTERIA FROM THE INTESTINAL TRACT

BY

LUTHER ALLEN BLACK

B.S., University of Illinois, 1924
M.S., University of Illinois, 1925

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1928

URBANA, ILLINOIS
1928

STANFORD UNIVERSITY LIBRARIES
WITHDRAWN

3781
I29B
UNCLASSED

A STUDY OF THERMOPHILIC BACTERIA FROM THE INTESTINAL TRACT

BY

LUTHER ALLEN BLACK

B.S., University of Illinois, 1924

M.S., University of Illinois, 1925

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1928

URBANA, ILLINOIS
1928

Reprinted from
Centralblatt für Bakteriologie, Abt. I, Vol. 75 (1928) 360-375

Digitized by the Internet Archive
in 2025

https://archive.org/details/studythermophili0000luth

Reprint prohibition.

A study of thermophilic Bacteria from the intestinal tract.

By **Luther A. Black** with **Fred W. Tanner**,
Department of Bacteriology, University of Illinois, Urbana, Illinois.

Although the importance of the study of thermophilic organisms has been recognized within recent years, only a very few investigations have been made where a large number of organisms were used. This is particularly true with thermophilic organisms from the intestinal tract. Bruini (8) has last conducted such an investigation of thermophilic bacteria from fecal sources. Early investigators have left us only a meager description of the salient characteristics of the organisms they described.

This investigation is one of a series of studies on thermophilic bacteria from the Department of Bacteriology of the University of Illinois. The present study was undertaken in order to determine the various types of thermophilic organisms present in the intestinal tract of humans and animals. It was desired to ascertain their distribution and number, and to obtain an exact cultural description of each of the species isolated, in order to render possible a comparison with such organisms from other sources. A large number of carbohydrates were used in fermentation studies in order to test this characteristic as a basis for sub-division of species. It was also desired to determine the resistance to heat of spores of these organisms.

Historical.

Only a brief resume of the investigations on thermophilic organisms from the intestinal tract will be given:

The first thermophilic organism isolated from this source was described as Bacillus thermophilus by Miquel (19). Miquel found this organism in air, rain water, river water, sewage, soil and in the intestinal tract of man and animals. The description given of this organism is meager and it is possible that the organisms isolated from the various sources are not identical. Bacillus thermophilus was described as an aerobic, non-motile rod, which formed oval terminal spores. Growth was secured from 42° to 72° C. This organism was not pathogenic to animals in sub-dermal, intravenous or intraperitoneal injections.

Globig (14) as quoted by McFadyen and Blaxall (17) and by Rabinowitsch (23) was not able to demonstrate thermophilic organisms in the feces of man and animals. Globig stated that when he inoculated potato slices with the intestinal contents of man, dog, guinea pig, rabbit, pigeon and mouse, and incubated these at 64° C., there was no growth or only a trace of growth visible in seven days. Samples from soil gave luxurious growth in two days. In a later series at 60° C., when a single colony was obtained upon a plate from rabbit feces, this was attributed to contamination of the sample by dust. However, when human or animal feces were streaked on potato and incubated at 50° C. there was frequently growth. Globig did not describe any organisms from fecal sources. — Sames (24) isolated thermophilic bacteria from feces but did not describe any species. — McFadyen and Blaxall (17) next isolated thermophilic organisms from the alimentary canal. In experiments with human, mouse, and hen feces, good growth was obtained in all cases at 60°—65° C. A sample of dysenteric feces also gave growth. Gelatin, agar, broth, Koch's peptone water and potato were used for isolation. All of the organisms examined were bacilli. Representative forms from various sources were studied more closely and at least twenty different species differentiated. These were all spore-bearing, many were motile, a few liquefied gelatin and some curdled milk. Mac-Fadyen and Blaxall stated that the term „thermophilic" strictly speaking, implied an organism that grew best at high temperatures, that could be cultivated from generation to generation at high temperatures, and that did not grow or only feebly, at low temperatures.

Rabinowitsch (23) examined the excrement of the horse, ox, cow, goat, rabbit, guinea pig, dog, mouse, pigeon, hen, duck, parrot, besides the entire digestive tract of man, a fish, frog, lizard and python. Thermophilic bacteria appeared more or less abundantly in each of these. Eight species were described. Bacilli 2, 4, and 8, formed central spores while 1, 3, 5, 6, and 7 formed terminal spores. These organisms were non-motile, facultative anaerobes, and were not pathogenic. They were separated on colony characteristics.

In a second paper McFadyen and Blaxall (18) among other sources, found thermophilic organisms in the intestine of man and horse, digestive tract of ducks, fowls, mice, rabbits, guinea pigs, frogs and snails, in fish, goldfish, carp, eel, whiting, and in oysters. Two organisms were described from animal dejecta. Bacillus I was non-motile, Gram negative and formed spores toward the center. Gelatin was liquefied, there was no indol, nitrates were not reduced, no action on milk. Bacillus II was non-motile, Gram positive, formed an oval spore towards end, produced indol, and reduced nitrates. These organisms reduced litmus and did not hydrolyze starch.

Tsiklinsky (26) investigated the meconium and feces of nursing infants of Moscow and Paris, as well as the feces of an adult. Twenty samples from infants in Moscow gave seven species, all Gram positive bacilli and non-pathogenic. Bacilli 1, 3, 4, 5, and 6 were obligate aerobes and obligate thermophiles. Bacillus 3 formed indol and had terminal spores, while Bacilli 4, 5, and 6 were reported as non-spore forming. Bacilli 2 and 7 were facultative aerobes and thermophiles, Bacillus 7 was motile, liquefied gelatin, and coagulated milk. From eighteen samples from infants in Paris four species of bacteria were isolated. These were spore-forming, Gram positive, facultative aerobes, grew at low temperatures and were not pathogenic. All except Bacillus 8 liquefied gelatin, Bacillus 9 formed central spores. One streptothrix was isolated from Paris. From eight samples at different times from the same adult, seven bacilli and one streptothrix were isolated. These bacilli were all Gram positive and only one, Bacillus 19, liquefied gelatin. Only bacilli 14 and 16 were facultative aerobes. Bacilli 14, 16 and 17 did not form indol, 13, 14, 15, and 16 were non-motile while 19 was motile. Bacilli 13, 15 and 18 formed terminal spores, 14 sub-terminal spores and 16 and 17 were reported as non-spore forming.

Bruini (8) examined ten samples of feces from infants and ten from one adult, following the methods of Tsiklinsky. From the feces of the adult Bruini isolated seven species of bacteria. These were all non-motile, spore-forming strict aerobes. All except Bacillus 3 were Gram positive. Bacilli 3, 4, 5, and 7 coagulated milk. None were pathogenic. Two Streptothrix were isolated from the adult. Samples from the infants gave three species of bacteria and three species of Streptothrix. Bacillus 11 was like bacillus 2 from the adult. These three bacteria were Gram positive, strict aerobes. Bacillus 10 was motile, 13 non-motile. Bacillus 13 was reported as non-spore forming. Bacilli 10 and 13 coagulated milk and were non-pathogenic.

Anitschkow (1) studied the approximate quantity of thermophilic bacteria in the intestinal canal of man, but did not describe any species. The results indicated that thermophilic bacteria occur in a relatively small quantity in the feces. Bergey (5) reported the characteristics of some thermophilic organisms from various sources. Guinea pig feces were found to contain two varieties of one type listed. In Bergey's Manual of Determinative Bacteriology (6) these types were named as new species and further characteristics were given. Taken all together, three types appeared in fecal sources. It is noteworthy that Type 4 liquefied gelatin in the 1919 description (5) while in the 1923 description (6) it is listed as Bacillus thermononliquefaciens, not liquefying gelatin. In the case of the other two organisms, the thermal death points do not agree in the two descriptions. These organisms are all motile, spore-forming, Gram positive bacteria, practically identical except for slight variation in temperature relation and gelatin liquefaction. The method used for gelatin liquefaction, three of four days incubation at 51° C. and then cooling, does not allow for slower growth or slower liquefaction.

Morrison (20) described six organisms from fecal sources. Cultures 53, 54, 55, from hog feces and 65, 66, and 67 from cow feces were described by Morrison and Tanner (21) under these numbers. Cultures 53, 54, 65, 66, and 67 were Gram positive rods, with peritrichic flagella and central spores. They were facultative anaerobes, liquefied gelatin, reduced nitrates without gas and were not chromogenic. They hydrolyzed starch, formed acid from dextrose and sucrose but none from lactose. Culture 55 differed from these only in being aerobic.

Experimental.

Isolation of Cultures. The cultures used in this investigation were isolated from three sources, namely, human, cow, and horse feces. Specimens of feces were taken aseptically as soon as possible after defecation. It was found that a sample of about 10 grams was most satisfactory to work with. Larger amounts occupied too large a volume when diluted. The specimen was placed in a sterile jar having a cork stopper, or else in a large tube with a cotton plug. Wooden tongue depressors, sterilized in separate manila envelopes, were found more satisfactory to obtain samples with than were cotton swabs. Specimens remained at room temperature until diluted. Sterile physiological salt solution was then added, 9 c. c. per gram of sample, and the solution poured into a glass-stoppered bottle containing glass beads. After adding 10 c. c. more of saline per gram of sample, the bottles were placed in a shaking machine and shaken moderately for ten minutes.

Anaerobic (inverted) plates, after the technic used by Krumwiede and Pratt (15), earlier by Marino (16) and aerobic plates were poured, using 1 c. c. and 0,1 c. c. of the feces emulsion. After incubation of the plates at 55° C. over night, each different type of colony in each sample was picked. A dissecting microscope was of aid in this. Colonies from aerobic plates were placed in tubes of broth. Colonies from anaerobic plates were picked into broth from which the oxygen had been expelled by boiling. Sterile melted paraffin was added to preserve anaerobic conditions. After incubation at 55° C. over night, tubes showing growth were subcultured both aerobically and anaerobically in broth. If subcultures from anaerobic tubes grew aerobically, they were discarded. Each culture was then replated to purify.

A series of three plates were used for replating each culture. Approximately 1 c. c. of sterile saline was placed in each Petri dish. A loopful of culture was added to the saline in the first plate and mixed. After flaming the loop, a loopful from the first plate was added to the second plate, and the procedure repeated from the second plate to the third. After incubation overnight, single colonies were again picked into broth.

From 18 samples of human feces 59 organisms were isolated; 14 samples of cow feces yielded 57 organisms and from 6 samples of horse feces, 26 organisms were isolated. Of these 142 organisms, 140 were bacilli and 2 were Streptothrix. Organisms were found in all samples examined except 1 sample from an infant. This specimen was only a trace upon a cotton swab and apparently was insufficient.

Anaerobic study. All cultures picked from anaerobic plates grew aerobically in broth. In one series of experiments, subcultures from anaerobic plates were made by pouring aerobic plates and one set of aerobic plates was exposed to the atmosphere and one set was placed in a Novy jar, the air exhausted by vacuum and pyrogallic acid and sodium hydroxide added. Both sets of plates contained growth. In another experiment deep tubes of agar, previously boiled and quickly cooled, were used. These tubes were inoculated with three dilutions of the growth from anaerobic broth tubes. A set of plates also were poured and part of these placed in a Novy jar, with vacuum, plus pyrogallic acid and sodium hydroxide solution. All organisms growing anaerobically proved to be facultative aerobes.

Quantitative study. Upon two occasions, plates were poured from known dilutions of feces, and the colonies upon aerobic plates were counted. The results are indicated in table I.

Table I.

Sample	Source	Grams of feces	Salt solution added c. c.	Per cent of dilution	Colonies per c. c.	Colonies per gram of feces
1	Beef cow 1	22	209	9,5	9,000	94,000
2	Horse 1	6	114	5	5,300	106,000
3	Beef cow 2	28	113	17.4	25,000	143,750
5	Horse 2	4	76	5	750	15,000
6	Horse 3	7	133	5	200	4,000
7	Horse 4	8	152	5	85	1,700
8	Horse 5	12	228	5	70	1,400
9	Beef cow 3	38	180	17.4	9,000	51,630
10	Beef calf 4	12	228	5	18,000	360,000

Sample	Source	Grams of feces	Salt solution added c. c.	Per cent of dilution	Colonies per c. c.	Colonies per gram of feces
1	Human 11	8	152	5	30	600
2	Child 12	3	57	5	1	20
3	Human 3	8	152	5	20	400
5	Human 4	3	57	5	60	1,200
6	Horse 6	77	133	5	2,000	40,000
7	Dairy calf 4	5	95	5	250	5,000
8	Dairy cow 6	12	228	5	180	3,600
9	Dairy calf 6.	12	228	5	1,000	20,000
10	Dairy calf 7.	7	133	5	9,000	180,000
11	Dairy calf 8.	7	133	5	700	14,000
12	Dairy calf 9.	9	171	5	650	13,000

Methods of study. The 1924 Descriptive Chart of the Society of American Bacteriologists was used to record the characteristics of the organisms studied. Practically all of the determinations called for on the chart were made. In addition to the carbohydrates listed on the chart, eleven other carbohydrates were used in fermentation studies. Thermal death point determinations were made upon twentyfive representative organisms, selected from as varied sources as possible.

The methods included in the Manual of Methods for the Pure Culture Study of Bacteria prepared by the Committee on Bacteriological Technic of the Society of American Bacteriologists were followed whenever possible. Revised methods including the issue of October 1927 were used. The incubation temperature for all reactions was 55° C. This temperature was secured in a large incubator built into a wall, having double doors, and heated by hot air from electric heating units regulated automatically by a thermostat. All determinations were made in duplicate and many reactions, especially any negative results, were repeated many times.

Morphology. The morphology of vegetative cells was determined from 24 hour cultures on agar slants, from which smears were prepared and Gram stained. The cells were measured by means of an ocular micrometer. With the exception of 2 Streptothrix isolated, all of the organisms were rods, isolated, in pairs and in all but five cases, in chains. The average size at this age was 0.6—0.7 × 2.5—3.0 microns. The ends of the cells were rounding. Capsules were not indicated and capsule staining was omitted. The presence, form, and size of endospores was determined from slides of week old cultures stained with Gentian Violet. Size was measured with an ocular micrometer. The location of the spore was usually determined from a Gram stained smear of a 48 hour old culture, but in some cases slides of other ages were necessary. All cultures formed spores. One culture formed central spores, nine terminal spores and in the remaining one hundred twenty-four cultures the spores were excentric.

For motility young broth cultures of six to twelve hours were found to be best. The majority of the bacteria were motile upon the first examination and further examination of those remaining showed them to be motile also. The Casares-Gil flagella stain, Galli-Valerio (13) as published by Plimmer and Paine (22) was used to stain for flagella. The technic used was substantially the same that Morrison and Tanner (21) found „gave very satisfactory results". Repeated attempts with this method failed to demonstrate flagella upon the motile thermophilic organisms used although on the control Proteus vulgaris, flagella were stained with great ease. Many modifications of technic, staining procedure, dilution of the mordant 1 : 1 as recommended by Thatcher (25), 1 : 2 as recommended in the Manual of Methods and 1 : 4 as used by Plimmer and Paine, and variation in the preparation of the culture were tried but flagella were never demonstrated. The Gram stain used was the Hucker modification. Upon slides prepared from 24 and 48 hour agar slant cultures, 124 bacteria were Gram positive, ten were Gram negative. The 2 Streptothrix were Gram positive.

Agar slant and broth cultures of 24 hours were employed for the description of cultural characters. None of the bacteria displayed any characteristic that would separate it from the others. Colonies on agar plates were examined macroscopically and microscopically. The colonies were typi-

cally circular, smooth, convex, with an entire edge and finely-granular internal structure.

Physiology. In the study of temperature relations, agar slants were inoculated with a loop of a broth culture and placed at five different temperatures. The amount of growth was recorded in 24 hours. All cultures grew at 25° C. within two days, the majority showing growth after one day. All cultures grew at 60° C. In only one case was better growth secured at another temperature than 55° C. The maximum temperature for growth was determined by placing such tubes in an incubator or in an oil bath. The majority of the bacteria had about the same maximum, 60°—62°, 62°, or 63° C. A few were able to grow at a higher temperature and two cultures, 135 and 142, grew at 75° C. but not at 76° C.

Shake cultures in dextrose agar were used to determine the relation to oxygen. All bacteria were facultative anaerobes, agreeing with the results secured during their isolation. Relation to the reaction of the media was not determined. None of the cultures except 1 Streptothrix were chromogenic.

Gelatin stabs were inoculated, incubated and each day cooled in the refrigerator and examined for liquefaction. Tubes not inoculated served as controls. Tubes not liquefying were kept for sixteen days before discarding. Only six cultures failed to liquefy gelatin, in all other cases the liquefaction was complete. Starch agar plates were streaked, incubated 24 hours, and flooded with iodine solution. Hydrolysis occurred with 121 organisms, 13 failed to hydrolyze starch. None of the bacteria formed indol in plain broth or dehydrated Bacto-Tryptophane broth within two days as evidenced by the Gore modification of the Ehrlich-Bohme technic as described in the Manual of Methods. Controls with Escherichia coli were positive. In one series the reagents were added directly to the tubes with negative results.

Bacto Lead Acetate Agar stabs were inoculated for the production of hydrogen sulfide. Hydrogen sulfide was formed by all the bacteria. All cultures except six reduced nitrates. Gas was never produced. Controls were always negative. Litmus milk made from Klim skimmed milk, and Bacto-Purple milk were used in a study of reactions in milk. The brom-cresol-purple milk was more satisfactory as an indicator of reaction. Only 3 bacteria failed to completely reduce litmus, two of these gave partial reduction. An acid curd with subsequent peptonization was common to 86 cultures. In 33 cultures only an acid curd was formed, fourteen only peptonized milk and one culture caused no change.

Carbohydrate fermentation. — Fermentation studies were carried out in liquid media. Plain broth to which brom-thymol-blue had been added, Baker (3) was used. The addition of 12 c. c. of a 0.2% solution of the indicator per liter of broth was satisfactory. Tubes containing 9 c. c. of the broth were sterilized. To these were added 1 c. c. of a 10% solution of the sugar, prepared aseptically and sterilized separately. The tubes were incubated for sterility before and after adding the sugar and before inoculation. In Table II the cultures are grouped as to their fermentation reactions. The largest group contains 58 bacteria, alike in action on 14 carbohydrates. If Inulin were disregarded, this group would comprise 88 bacteria, and were Rhamnose, the other unsatisfactory carbohydrate omitted, 102 of the 134 bacteria react alike on 12 carbohydrates.

Table II. Carbohydrate Fermentation.

	Glucose	Levulose	d-Galactose	d-Mannose	l-Arabinose	l-Xylose	Rhamnose	Maltose	Lactose	Sucrose	Raffinose	Melizitose	Dextrin	Inulin	Cultures	Total
I	A	A	A	A	A	A	—	A	—	A	A	—	A	—	1, 3—6, 8, 21—23, 36—40, 45, 47, 50—52, 54, 59, 66, 69, 70, 72, 79, 83—85, 95, 97, 99, 100, 102, 103, 107, 111, 112, 114, 116, 119, 120, 123, 129, 131, 132, 136, 137, 139, 143, 146, 151, 152, 155, 156, 158, 160, 161	58
Ia	A	A	A	A	A	A	—	A	—	A	A	—	A	A	7, 41—43, 46, 57, 58, 60—63, 65, 71, 73—75, 78, 80, 86, 88—91, 101, 113, 117, 118, 124, 125, 140	30
II	A	A	A	A	A	A	A	A	—	A	A	—	A	—	96, 98, 105, 110	4
IIa	A	A	A	A	A	A	A	A	—	A	A	—	A	A	92, 93, 94, 109, 122, 126, 127, 149, 150, 153	10
III	A	A	A	A	A	A	—	A	A	A	A	—	A	—	49, 121	2
IIIa	A	A	A	A	A	A	—	A	A	A	A	—	A	A	56, 115	2
IV	A	A	—	A	A	A	—	A	—	A	A	—	A	—	2, 138, 144	3
IVa	A	A	—	A	A	A	—	A	—	A	A	—	A	A	9, 82	2
V	A	A	A	A	A	A	—	A	—	A	—	—	A	—	64, 68, 157	3
VI	A	A	A	A	A	A	—	A	—	A	A	A	A	O	108	1
VII	A	A	A	A	A	A	A	A	A	A	A	A	A	A	25, 44, 48, 67, 145, 147, 148, 154	8
VIII	A	A	A	A	A	A	A	A	—	A	A	A	A	A	135	1
IX	A	A	A	A	A	A	A	A	A	A	A	—	A	A	87, 162	2
X	A	A	A	A	A	A	—	A	A	A	A	A	A	A	142	1
XI	A	A	—	A	A	A	A	A	A	A	A	—	A	A	133, 134	2
XII	A	A	A	—	A	A	—	—	—	—	—	—	—	—	141	1
XIII	—	A	—	—	—	—	—	—	—	—	—	—	—	—	104, 106, 159	3
XIV	—	—	—	—	—	—	—	—	—	—	—	—	—	—	81	1

A = acid O = neutral — = alkaline

Table III shows the organisms grouped as to their index numbers. Characteristics not determined are designated U.

Thermal Death Points. — For this study, twenty-five representative organisms were selected from as many sources as possible. The method followed was a modification of the Bigelow and Esty (7) method, essentially the same as used by Morrison and Tanner (21). Before heating, however, the tubes were placed in a boiling water bath for 15 seconds, then in the oil bath for 15 seconds before recording the time. The temperature of the oil bath was previously increased a trifle for temperatures over 100°. Observations were made 24 and 48 hours after the plates were poured for the determination of sterility. Two days seemed ample time for growth to appear. The number of spores and the time to destroy the spores are shown in Table IV. „Skips“ in thermal death points were not troublesome. In the great majority of the determinations there were no skips. When skips occurred they were almost at the thermal death point and usually the growth at times above the skips consisted of only one or sometimes a very few colonies. By use of the plate method for the determination of the sterility of the heated spore suspension, these slight

differences were detected. The reaction of the medium varied from a pH of 6.8 in the flasks to 6.8 to 7.4 in the heated suspensions. Determinations were made to within two minutes at 100° C., up to 220 minutes; within one minute at 105° C. up to 60 minutes and at 110° C. up to 180 minutes; and within onehalf minute at 115° C. up to 30 minutes and at 120° C. up to 20 minutes.

Table III. Classification of Organisms by Index Numbers.

Class	Index-Number	Culture Number	Total
I	52 U 1—U 2120—1202	1—9, 21—23, 26—43, 45—47, 50—52, 54, 56—66, 68—74, 78—80, 82—88, 88—103, 105, 107, 109—114, 118—120, 122—127, 129, 131, 132, 136—140, 143, 144, 146, 149—153, 155—158, 160, 161	110
II	52 U 1—U 2120—1222	44, 87, 115, 121, 154, 152	6
III	52 U 1—U 2120—0202	75	1
IV	52 U 1—U 2020—0222	25, 147	2
V	52 U 1—U 2100—1222	49	1
VI	52 U 1—U 2100—0000	81	1
VII	52 U 1—U 2100—0222	148	1
VIII	52 U 1—U 2000—0222	67	1
IX	52 U 1—U 2000—1222	145	1
X	52 U 0—U 2120—1222	142	1
XI	52 U 0—U 2120—0202	108	1
XII	52 U 0—U 2120—0222	133, 134	2
XIII	52 U 0—U 2120—0000	104, 106	2
XIV	52 U 0—U 2020—1202	135	1
XV	52 U 0—U 2020—1222	48	1
XVI	52 U 0—U 2100—0200	141	1
XVII	51 U 0—U 2120—0000	159	1

With regard to cultures 135 and 142, determinations at 100° C. were made at intervals up to 3 hours in the first series. As the spores were not killed another series was kept at 100° C. up to 4½ hours. Since growth likewise occurred in all cases, a series was kept at this temperature for 8 hours, later another series was kept from 7 to 13 hours and still later from 12 to 24 hours, withdrawing a tube each hour from the oil bath. As growth still occurred, although greatly reduced, after 24 hours heating of the spore suspension at 100° C. another spore suspension was prepared. A series of tubes were heated from 24 to 36 hours withdrawing a tube each hour, with the results shown in Table IV.

Streptothrix. — Organism 55, a Streptothrix, was found once in a specimen of human feces. The filaments were from 0.4 to 0.9 microns in diameter and varied in length from short segments to very long filaments. The filaments were straight, curved or winding and appeared to have true branching. The branches or filaments sometimes ended in ellipsoidal bodies 1.5—2.0 × 2.0—3.0 microns. Upon agar slants the growth was scanty, consisting of flat dull chalky-white isolated colonies. In broth the growth was under the form of isolated, white cotton-like spheres or flakes at the bottom of the tube. This organism liquefied gelatin, but did not reduce nitrates, hydrolyze starch, form indol or hydrogen sulfide. It reduced litmus and curdled milk with peptonization. Glucose, levulose, galac-

tose, mannose, arabinose, xylose and melizitose were fermented with acid formation. The fermentation of rhamnose and maltose was doubtful.

Table IV. Thermal Death Points in Minutes at Temperatures Indicated.

Culture	Spores /cc.	100° C.	105° C.	110° C.	115° C.	120° C.
1	2,720,000	32	10	4	3	1.5
3	37,000	19	7	4	2.5	2.5
37	39,300	9	4	2	1.5	1.5
44	42,000	12	3	2	1.5	1.5
48	60,800	48	16	5	3	3
56	9,800	12	6	3	1.5	1
67	5,440,000	225	70	14	5	2.5
68	56,100	28	7	4	2	1.5
75	8,000,000	37	11	5	3	2
83	8,200,000	60	12	5	3	2
101	9,200	4	2	2	1	1
104	82,800	58	13	5	3	2
106	400,000	154	20	6	4.5	2.5
108	7,000	14	5	3	1.5	1
115	200,000	35	8	4	2.5	1.5
120	112,000	22	7	4	2.5	2
133	45,600	16	6	3	1.5	1.5
134	36,000	11	6	3	1.5	1.5
135	620,000	24 hrs.[1])	12 hrs.[1])	3 hrs.[1])	60 [1])	25
141	2,700,000	300	126	16	5.5	2
142	6,400	25 hrs.[1])	12 hrs.[1])	3 hrs.[1])	60 [1])	27 [1])
145	2,200	16	6	4	1.5	1.5
147	44,600	14	6	3	2	2
154	2,250	9	2.5	2	1.5	1
159	3,960	120	25	8	3.5	2.5
Another spore suspension of 135 and 142 gave the following results						
135	4,300	27 hrs.	12 hrs.	4¼ hrs.	90	25
142	3,200	29 hrs.	12 hrs.	5 hrs.	90	25

[1]) indicates survived

The other Streptothrix, organism 130, was isolated from the feces of a horse. The filaments were much the same as 55 except the ends tended to thicken instead of an ellipsoidal body. The filaments were sometimes granulated in appearance. Upon agar slants the growth was moderate, filiform, convex and glistening. There was a tendency to isolated colonies. The color was yellow and a musty odor was sometimes noticed. In broth there was often a ring but usually the growth was at the bottom under the form of cotton-like flakes or spheres. This organism liquefied gelatin, hydrolyzed starch, and formed hydrogen sulfide. Nitrates were not reduced and indol not formed. Litmus was reduced and an acid curd formed in milk. Glucose, levulose, galactose, arabinose, xylose, were fermented with acid production. Anaerobic growth occurred.

Discussion.

A comparison of the organisms isolated with reference to their source, seemed to indicate that this made no difference in their characteristics. The majority from each source were placed in the same index number and each source had representatives in the 16 other index numbers.

Thermophilic organisms were present in all specimens of feces examined from the 3 sources and at all ages. As shown in Table I, the number of thermophilic bacteria varied from 20 to 360,000 per gram. The lowest number was from a small child. The next lowest number found was 400, also from a human source. In the few specimens examined the highest number of bacteria per gram were found in cattle feces, the lowest in humans. The small number of thermophilic organisms in the human intestinal tract compares favorably with the results obtained by Anitschkow (1) as previously related.

The bacteria isolated were characteristically motile, sporeforming rods, facultative anaerobes. The majority were Gram positive. In general these organisms liquefied gelatin, hydrolyzed starch, and reduced nitrates. Glucose and sucrose were fermented. None of the organisms were chromogenic. Litmus was also reduced.

In connection with the failure to demonstrate flagella upon motile thermophilic organisms, the results of Feirer (12) are of interest. With motile thermophilic organisms isolated from soil, Loeffler's, and Plimmer and Paine's methods were used repeatedly by Feirer to demonstrate flagella but without any definite result. The flagella of Bacillus coli were stained successfully by both methods. While Feirer considered that there may be some difference chemically in the flagella, due to adaptation to high temperature, the possibility was advanced of motility due to a contractile property of the cytoplasm. Eckford (10) could not demonstrate flagella on thermophilic bacteria from milk, although many of the strains were motile. Werkman and Weaver (28) stated that the organisms they studied were motile and the motility suggested peritrichic flagella. However flagella were not demonstrated by staining methods.

On the other hand, Bergey (6) described the motility of thermophilic organisms studied as due to peritrichic flagella. Incidentally no mention was made of this in the 1919 (5) description of these organisms. Since Morrison and Tanner (21) were able to demonstrate flagella by use of Plimmer and Paine's method, it is felt that further study of staining technic and application to thermophilic organisms of various modifications, may enable the demonstration of flagella. This should be done before assuming with Feirer (12) the possibility that the motility may instead be due to a contractile property of the cytoplasm.

Upon dextrose agar there seemed to be little that was characteristic about the colony of any of these thermophilic bacteria. Most of the investigators who have described marked colony characteristics have used potato in some form as the medium. Chromogenesis likewise seemed to be absent upon the dextrose agar. McFadyen and Blaxall (18) obtained similar results when agar was used.

No strict anaerobic thermophiles were isolated. All organisms isolated by both anaerobic and aerobic methods were facultative anaerobes. Veillon (27) described thermotolerant organisms which were supposed to be strict anaerobes, isolated from manure of various origins. Only 1 of these organisms was spore-bearing. All 3 could develope between twenty and fifty-eigth degrees. They were not pathogenic. Damon and Feirer (9) described some organisms that were termed obligate anaerobic thermophiles. These were isolated from horse manure. None of the cultures

were pathogenic for guinea pigs. Werkman and Weaver (28) proposed the name Clostridium nigrificans for a thermophilic spore-forming organism they described as anaerobic. This organism was isolated from spoiled canned sweet corn and later from soil and horse manure. It was not pathogenic.

The first investigator to attribute pathogenicity to thermophilic organisms was Bruini (8). Upon injection of a guinea pig with one cubic centimeter of a three day culture, death appeared after six to eighteen days. Bruini believed that these cases of death were due to toxic products elaborated by the bacteria. Ascione (2) described a thermophilic Streptothrix isolated by De Blase from the Agnano hot springs. This organism began to grow profusely at 45 degrees Centigrade, grew luxuriantly at 55 degrees and even more at 60 degrees. It was the only organism recovered from the water at 55 degrees. When inoculated into a guinea pig this Streptothrix caused death in from 5 to 15 days with serious toxic symptoms. This action seemed due to a hemolytic poison. All other investigators of thermophilic organisms from any source that have tried their organisms for pathogenicity, have found the organisms were not pathogenic for the laboratory animals used.

Barlow (4) stated that a healthy man with empty stomach masticated and swallowed three teaspoonfulls, over 15 grams, of corn in which thermophilic organisms had grown. Although the taste was unpleasant, it did not cause decided nausea and no further effects were observed. Barlow stated that these thermophiles did not grow at blood temperature of mammals and apparently growth at high temperatures in canned corn did not produce toxins. More recently Werkman and Weaver (28) fed a thermophile described as anaerobic to man, guinea pig, mouse, rat and rabbit without effect. Thermophilic bcateria are frequently present in milk yet they have never been reported as causing any harm.

The descriptions given by early investigators of the organisms isolated are insufficient in many cases for a proper comparison. Bacillus thermophilus as described by Miquel (19) was an aerobic rod forming oval terminal spores. The thermophiles described by Rabinowitsch (23) were non-motile bacilli. Bacilli 2, 4, 8, formed central spores while 1, 3, 5, 6, 7, formed terminal spores. These organisms were divided on the basis of colony characteristics. Of the two organisms from animal dejecta described by McFadyen and Blaxall (18) Bacillus I, as far as the characteristics are given, corresponds to Class XVI of the present investigation, except it is non-motile. Bacillus II similarly corresponds to Class IV except it is non-motile and forms indol.

Tsiklinsky (26) isolated seven organisms from infants in Moscow, four from infants in Paris and seven bacilli from an adult, at various times. A Streptothrix was also isolated once in Paris and another from the adult. The characteristics of these bacilli described by Tsiklinsky were not detailed enough to compare them to the cultures of the present investigation. Bruini (8) isolated seven bacteria from the feces of an adult and three from the feces of infants in Turin. One Streptothrix was isolated from the adult and 3 from infants. These bacteria were likewise described insufficiently to enable comparison. Bergey (5) described a Type 4 that, as far as the characteristics are given, coincides with Class I or II. It does not grow at low temperasures, however, and in 1923, Ber-

gey (6) described it as not liquefying gelatin, which would remove it from Class I or II. As far as the characteristics are given, Bacillus thermoliquefaciens as described by Bergey (6) corresponds to Class III. The organism Bergey described as Bacillus thermotranslucens in so far as the characteristics are given, seems to correspond to Class VIII or IX, depending upon the doubtful starch hydrolysis reaction.

Morrison (20) isolated six organisms from hog and cow feces. The index numbers of cultures 53, 54, from hog feces and 65, 66, 67, from cow feces, as described by Morrison and Tanner (21), correspond to Class I of the present investigation except they were described as bearing central spores. However in the Descriptive Chart used by Morrison, spore location was either central or polar, while the chart now used classifies spore location as central or excentric to terminal. The possibility exists that the organisms mentioned may have had excentric spores as those of Class I. The organism 55 from hog feces differed from the others only in being described as a strict aerobe instead of facultative. The criteria then used was growth in the closed arm of a fermentation tube. Perhaps had another method for the determination of oxygen relations been used, the „strict" aerobes may have been facultative anaerobes.

Culture 55, a Streptothrix, closely resembles Streptothrix 12 of Tsiklinsky (26). Streptothrix 20 of Tsiklinsky is somewhat similar but does not liquefy gelatin, change milk, or grow at low temperatures. Streptothrix 8, 9, and 12 of Bruini (8) are not described fully enough as to physiological characteristics to enable differentiation. The Streptothrix 14 of Bruini differed slightly in having yellowish colonies and not growing at 37 degrees. Streptothrix 15 of Bruini was not observed to grow on plain agar and did not coagulate milk. Morphologically all of these organisms are similar to culture 55. The characters upon which Tsiklinsky and especially Bruini separated their species, seemed to be of minor importance, reactions subject to variation or negative reactions. Culture 130, a Streptothrix, appears to be different from any organism whose description has been found in the literature.

The fermentation reactions of carbohydrates were not always satisfactory. It was not infrequently observed that an organism might produce acid in a tube within 24 hours and then the reaction of the media would change to neutral or alkaline. In some cases acid might again appear at a later time. The opposite situation was also noted, first alkaline, acid sometime later, and perhaps a return to alkaline, or more often, a continuation of the acid reaction. Tubes have been observed daily for ten days or two weeks and such changes noted. These changes occurred especially with Inulin, Rhamnose and to a lesser extent with Raffinose. This did not interfere with the use of Raffinose as all but seven organisms fermented this sugar. Acid production in this case was rapid and could be recorded within 16 to 24 hours in most cases. After this time many of the organisms changed the reaction to alkaline. Some were persistently acid. With Inulin and Rhamnose the effect was different. Inulin was fermented by only 57 organisms, Rhamnose by only 27 organisms. There was much fluctuation in acid production with these two carbohydrates. This variation appeared, as stated before, in a particular tube at various ages

and in duplicate tubes or tubes of another series. Tubes showing reactions undoubtedly acid were repeated and tubes with negative or doubtful reactions were repeated many times. With certain sugars the control tubes tended to become acid after several days incubation at 55 degrees centigrade. This was apparently due to the effect of the high temperature, as this did not occur at room temperature. The indicator also tended to change after prolonged incubation, apparently broken down by the effect of the heat. Sugar reactions were of little help in differentiation of the thermophiles studied.

All of the organisms isolated grew from 25 degrees to 60 degrees centigrade. Growth was slower at the lower temperatures. The majority of these bacteria quickly reached their maximum after 60 degrees. There were two cultures able to grow at 75 degrees centigrade. These two organisms were able to form resistant spores as was shown in Table IV.

In the spore suspensions prepared for the thermal death points, the number of spores per cubic centimeter varied to a striking degree. As shown in Table IV, there was a marked variation among individual cultures. There was also a considerable variation among preparations of the same culture. The second suspension prepared of culture 135 contained 4300 spores per c. c. compared to 520,000 spores in the first suspension. Two cultures were inoculated into flasks but yielded only 16 and 24 spores, respectively, per cubic centimeter. These two organisms were not used for thermal death point determinations. Apparently the conditions that obtained allowed a great variation in the number of spores produced. Feirer (12) after incubating cultures at 55 degrees for 7 days, then incubated for 2 days at 65 degrees. Esty and Williams (11) concentrated spore suspensions when the number was not satisfactory. It seems, then, that individual cultures may vary in their ability to, or necessity to, form spores under a given set of conditions. This initial concentration of spores has an effect upon the thermal death point but not more so than the resistance of individual organisms.

Eckford (10) in a study of thermophilic bacteria from milk stated that „The results indicate . . . that among the thermo-tolerant organisms, there is a direct relation between high maximum growth and a greater heat resistance of spores. Spores of true thermophiles were found to have a higher thermal death point than those of thermotolerants. Thermophiles with a maximum thermal range of 75 degrees produced spores which could withstand 120 degrees for 15 minutes in the De Khotinsky oil bath, whereas thermotolerants with the same high maximum could withstand 115 degrees for the same time." Bergey (5) stated that „The true thermophiles resisted a temperature of 100 degrees centigrade to an unusual degree varying from 2 to 8 hours, so that it appears probable that the optimum temperature for growth is related to the heat resisting powers of the spores. The facultative thermophiles with their lower optimum temperature for growth also showed a lowered resistance to the boiling temperature. This group of bacteria was killed in from one-fourth to one hour at 100 degrees centigrade."

While a direct relation was found between high maximum growth and greater heat resistance of spores, as pointed out by Eckford (10), the results of the present investigation did not agree with the distinction Eckford made between true thermophiles and thermotolerant organisms. The term „true thermophile" as used by Eckford included only those organisms that would not grow below 42 degrees. In contrast to such results two organisms isolated in the present investigation, capable of growth at

25 degrees as well as 75 degrees, produced spores that withstood 25 minutes at 120 degrees and 60 minutes at 115 degrees, considerably more resistant than the so-called true thermophiles described by Eckford. In connection with the statement of Bergey (5) that true thermophiles resisted 100 degrees for 2 to 8 hours, these resistant thermotolerant or facultative organisms of the present investigation resisted 100 degrees centigrade for 24 hours or longer. Bergey found such facultative thermophiles were killed in 15 to 60 minutes at 100 degrees.

It seems that there can be as great resistance among thermophiles capable of growth at room temperature as among „true thermophiles". Possibly many of the so-called true thermophiles could grow at lower temperatures if they were given more time and perhaps a more favorable medium, reaction or other favoring condition. In this connection it seems that the use of the term „thermophile" to mean organisms growing best at high temperatures, has something to recommend it over the present tendency of investigators to fix an arbitrary minimum temperature for „true" or „strict" thermophiles and term thermophilic organisms growing below this arbitrary minimum „facultative" or „thermotolerant". Such terms might be used to refer to different optimum temperatures and yet not arbitrarily set a minimum temperature of growth for thermophilic organisms.

Summary.

1. Thermophilic organisms were readily isolated from the specimens of human, cow and horse feces. No appreciable difference was noted in the characteristics of thermophiles isolated from various sources. The number of thermophilic bacteria per gram of feces varied considerably among the different specimens examined. Strict anaerobic thermophiles were not found. — 2. Of the 142 thermophilic organisms isolated, 140 were bacteria and 2 were Streptothrix. The cultural characteristics of the thermophilic bacteria were not distinctive None of the bacteria were chromogenic. — 3. All of the bacteria studied were spore-bearing, motile rods and all were facultative anaerobes. — 4. Flagella upon thermophilic bacteria seem to be more difficult to stain than those upon ordinary „mesophilic" bacteria. — 5. Of the 134 strains of thermophilic bacteria studied 124 were Gram positive. Excentric spores were formed by 124 cultures, 9 cultures formed terminal spores and 1 produced central spores. Of these bacteria, 110 were grouped under the same index number. The remaining 24 cultures were divided among 16 other index numbers, upon differences by Gram stain, gelatin liquefaction, nitrate reduction, starch hydrolysis and variation in sugar fermentations. — 6. Culturally the bacteria were active, 128 liquefied gelatin and 100 of these also peptonized milk. Nitrates were reduced without gas production by 128 bacteria,

121 organisms hydrolyzed starch and 133 reduced litmus. Indol was not formed by any organism while all cultures produced hydrogen sulfide. — 7. The thermophilic bacteria isolated were strongly fermentative upon most of the 14 carbohydrates used. Some lactose fermenters were found. Gas was not formed in any case. The fermentation reactions were not satisfactory as a means for the subdivision of species. — 8. All of the bacteria grew at temperatures of 25 degrees to 60 degrees centigrade. Many organisms grew at a slightly higher temperature, two cultures grew even at 75 degrees. — 9. The thermal death points seemed to vary for each species, although they were also influenced by the concentration of spores. There was a considerable variation in the number of spores produced by the various organisms under the same conditions. The number of spores produced by the same organism also varied upon repetition under similar conditions. — 10. The spores of two organisms resisted 100 degrees centigrade for over 24 hours. Apparently the spores of thermophiles capable of growth at room temperature, may be as resistant to heat as spores of the so-called „true" thermophiles, that have been reported to not grow at such low temperatures. Also the spores of thermophilic organisms isolated directly from nature seem as resistant to heat as spores of thermophiles that may have been „selected" for heat resistance, by isolation from sources previously heated or processed.

References.

(1) Anitschkow, N. N., Zur Frage über die Rolle der thermophilen Bakterien im Darmkanal des Menschen. (Centralbl. f. Bakt. Abt. I. Orig. Bd. 41. 1906. S. 326—332, 426—432.) — (2) Ascione, G., A pathogenic thermophilic Streptothrix. (Ann. d'Ig. T. 37. 1927. p. 429—437. [Original not seen.] Abstract in Journ. Amer. Med. Assoc. T. 89. 1927. p. 1729.) — (3) Baker, H. R., Substitution of bromthymol-blue for litmus in routine laboratory work. (Journ. Bact. Vol. 7. 1922. p. 301—305.) — (4) Barlow, B., A spoilage of canned corn due to a thermophilic bacterium. [Thesis.) University of Illinois. 1913. — (5) Bergey, D. H., Thermophilic bacteria. (Journ. Bact. Vol. 4. 1919. p. 301—306.) — (6) Bergey, D. H., In Bergey's Manual of Determinative Bacteriology William and Wilkins Company. 1923. — (7) Bigelow, W. D., and Esty, J. R., The thermal death point in relation to time of typical thermophilic organisms. (Journ. Infect. Dis. Vol. 27. 1920. p. 602—617.) — (8) Bruini, G., Über die thermophile Mikrobenflora des menschlichen Darmkanals. (Centralbl. f. Bakt. Abt. I. Orig. Bd. 38. 1905. S. 177—185, 298—306.) — (9) Damon, S. R., and Feirer, W. A., Anaerobic sporulating thermophiles. (Journ. Bact. Vol. 10. 1925. p. 37—46.) — (10) Eckford, Martha O., Thermophilic bacteria in milk. (Amer. Journ. Hyg. Vol. 7. 1927. p. 201—221.) — (11) Esty, J. R., and Williams, C. C., Heat resistance studies. A new method for the determination of heat resistance of bacterial spores. (Journ. Infect. Dis. Vol. 34. 1924. p. 516—528.) — (12) Feirer, W. A., Studies on some obligate thermophilic bacteria from soil. (Soil Science. Vol. 23. 1927. p. 47—56.) — (13) Galli-Valerio, B., La méthode de Casares-Gil pour la coloration des cils des bacteries. (Centralbl. f. Bakt. Abt. I. Orig. Vol. 76. 1915. S. 233—234.) — (14) Globig, Dr., Über Bakterien-Wachstum bei 50—70°. (Ztschr. f. Hyg. Bd. 3. 1888. S. 294—321.) — (15) Krum-

wiede, C., and Pratt, Josephine, Fusiform bacilli. Isolation and cultivation. (Journ. Infect. Dis. Vol. 12. 1913. p. 199—201.) — (16) Marino, F., Methode pour isoler les anaerobes. (Ann. de l'Inst. Past. Vol. 21. 1907. p. 1005—1008.) — (17) McFadyen, A., and Blaxall, F. R., Thermophilic bacteria. (Journ. Path. a. Bact. Vol. 3. 1894. p. 87—99.) — (18) McFadyen, A., and Blaxall, F. R., Thermophilic bacteria. (Trans. of the Jenner Inst. of Prevent. Med. 2nd series. 1899. p. 162—187.) — (19) Miquel, P., Bull. de la Statique municipale de Paris Again published 1888 Monographie d'un bacille vivant au-dela-de 70° Centigrade Ann. Micrographie. Vol. 1. 1879. p. 3—10.) — (20) Morrison, Lethe E., Studies on thermophilic bacteria. (Thesis for degree of Doctor of Philosophy, University of Illinois 1922.) — (21) Morrison, Lethe E., and Tanner, F. W., Studies on omit thermophilic bacteria. (Botanical Gazette. Vol. 77. 1924. p. 171—184.) — (22) Plimmer, H. C., and Paine, S. G., A new method for the staining of bacterial flagella. (Journ. Path. a. Bact. Vol. 24. 1921. p. 286—288.) — (23) Rabinowitsch, Lydia, Über die thermophilen Bakterien. (Ztschr. f. Hyg. Bd. 20. 1895. S. 155—164.) — (24) Sames, T., Zur Kenntnis der bei höherer Temperatur wachsenden Bakterien- und Streptothrix-Arten. (Ztschr. f. Hyg. Bd. 33. 1900. S. 313—362.) — (25) Thatcher, Lida M., A modification of the Casares-Gil flagella stain. (Stain Technology. Vol. 1. 1926. p. 143.) — (26) Tsiklinsky, Mlle. P., Sur la flore microbienne thermophile du canal intestinal de l'homme. (Ann. de l'Inst. Past. Vol. 17. 1903. p. 217—240.) — (27) Veillon, R., Sur quelques microbes strictement anaerobies. (Ann. de l'Inst. Past. Vol. 36. 1922. p. 422—439.) — (28) Werkman, C. H., and Weaver, Helen J., Studies in the bacteriology of sulphur stinker spoilage of canned sweet corn. (Iowa State College Journ. of Science. Vol. 2. 1927. p. 57—67.)

VITA

Luther Allen Black graduated from the Decatur, Illinois, High School in 1920, and received the degree of Bachelor of Science in Agriculture from the University of Illinois in 1924. He received an appointment as Scholar in Bacteriology at the University of Illinois for 1924-1925, and received the degree of Master of Science in Bacteriology in 1925. He was an Assistant in Bacteriology at the University of Illinois during 1925-1926.

From June 1926 until February 1927, he was Bacteriologist in the Diagnostic Laboratory of the Illinois State Department of Public Health. He resigned this position to resume graduate study at the University of Illinois. During the summer of 1925 and of 1927, he was Research Bacteriologist in Dairy Bacteriology, Agriculture Experiment Station, University of Illinois. He was also Bacteriologist for the McKinley Memorial Hospital of the University of Illinois.

He is a member of the Society of American Bacteriologists, American Public Health Association, Illinois Medical Laboratory Association, Alpha Zeta, Phi Sigma, and an active member of Sigma Xi.

Publications:

Undulant Fever as a Public Health Problem
Thomas G. Hull, Ph.D., and Luther A. Black, M.S.
Jour. Am. Med. Assn., 1927, 88, 463-464.